18952

STIC-Biotech/ChemLib

From: Bakalyar, Heather
To: STIC-Biotech/ChemLib
Subject: 08/656811
Date: Monday, September 08, 1997 2:06PM

Heather Bakalyar
1818
305-7143

1. Dash et al, Molecular Brain Research 39(1-2) 1996 43-51
2. Nguyen et al Science Aug 19, 1994 265(5175) p1104-7
3. Alberini et al, Assn N Y Acad Sci Jun 30 1995 758 pages 261-86
4. Alberini et al, Cell, March 25, 1994, 76(6) 1099-14
5. Kaang et al Neuron March 1993 10(3) pages 427-35
6. Dash et al, Nature Jun 21 1990, 345 (6277) pages 718-21
7. Bergold et al PNAS 1990 87/10 pages 3788-3791
8. Olds et al New Biol 1991 3/1 pages 27-35 ISSN 1043-4674
9. Brockeroff et al FASEB J 4(4) 1990 A899
10. Thank you!

9/9
ND

NIH
9-10-97
904

A ROLE FOR PROTEIN KINASE C IN ASSOCIATIVE LEARNING

James L. Olds* and Daniel L. Alkon

Recent work suggests that protein kinase C (PKC), an enzyme that has a critical role in the regulation of cell growth and differentiation, also participates in the sequence of molecular events that underlie learning and memory. By means of electrophysiological, biochemical, and neuro-imaging methods it has been demonstrated that, in the brain, the distribution of PKC changes as a result of memory storage. The changes in distribution occur within the same ensembles of nerve cells that are necessary for the acquisition and performance of various learning tasks in several species. Here we review the data pertaining to a model that has been proposed to account for the participation of PKC as a molecular signal for cotemporal synaptic input during associative learning.

Received August 21, 1990; revised November 9, 1990

The enzyme protein kinase C (PKC) is a calcium-lipid dependent protein kinase that catalyzes the phosphorylation of specific substrate proteins. First characterized by Nizhisuka in 1979, PKC has since been found to have a ubiquitous role in regulating cellular function in most eukaryotic systems (Nishizuka, 1988; Alkon and Rasmussen, 1988). Unlike most kinases, PKC is characterized by requiring both an appropriate lipid milieu and a Ca^{2+} signal for physiological activation (Hannun et al., 1985; Morimoto et al., 1988). Recently, it has been found to play an important role in associative learning (Olds et al., 1989; Olds et al., 1990; McPhie et al., 1990; Sharenberg et al., 1990) as assessed by quantitative autoradiographic techniques. Other methodologies (Bank et al., 1988; Wehner et al., 1990a,b; Nelson et al., 1987) have tended to confirm the evolving hypothesis that PKC activation in the brain may serve as a nexus in the chain of neuronal events that leads to the laying down and storage of memory.

We discuss here a model in which PKC serves as an intracellular molecular signal for concurrent presynaptic input, probably in concert with other critical enzymatic regulators. Thus, in the proposed model, PKC is ideally situated to act as a neuronal indicator that two environmental stimuli are occurring near one another in the time domain. Such a function could have important theoretical implications for the development of artificial neuronal networks (Alkon et al., 1990). Via neuronal tertiary-messenger cellular pathways, the PKC system could eventually lead to extremely long-term, learning-specific changes in neuronal gene expression and morphology.

THE PKC MOLECULE

Molecular Profile

Depending on the species from which PKC is isolated, the molecular weight of the enzyme is between 80,000 and 90,000 (Nishizuka, 1988). The corresponding gene is a member of a large family and at least seven different isoforms of the gene have been isolated (for review see Nishizuka, 1988). There is a close correspondence between the different genes for PKC that have been isolated and the different isozymes of the protein (Huang et al., 1987; Woodgett et al., 1987; Huang FL et al., 1988; Huang KP et al., 1988; Nishizuka, 1988; Stichel et al., 1988; Nakabayashi et al., 1988; Yoshida et al., 1988; Hidaka et al., 1988; Huang FL et al., 1989; Huang KP et al., 1989; Roth et al., 1989; Saito et al., 1989). At least one of the isozymes (corresponding to the gamma isoform of the gene) is specific to the brain and is highly enriched in the mammalian hippocampus (Stichel et al., 1988; Roth et al., 1989; Nishizuka, 1988). Several of the other isozymes are also highly prevalent in brain tissue and, on the basis of hybridization studies, appear to exhibit distinctive neuroanatomical distributions (Stichel et al., 1988).

Laboratory of Molecular and Cellular Neurobiology, National Institutes of Health, Bldg 9, Room 1W125, Bethesda, MD 20892.
*To whom correspondence should be addressed.

1043-4674/91/0301-0001$5.00/0

KEY WORDS: learning and memory/protein kinase C/associations/hippocampus/second messenger systems/rat/rabbit/nictitating membrane/*Hermissenda crassicornis*

Membrane Association

Because PKC belongs to a class of proteins that are amphitrophic, that is, composed of regions which are both hydrophobic and hydrophillic (Brumfeld and Lester 1990; Lester et al., 1990), the localization of the enzyme in the membrane milieu has been thought to reflect the enzyme's state of activation. Early studies clearly demonstrated that the activity of the enzyme dependended upon the phospholipids phosphotidylserine (PS) and phosphotidyl choline (PC) (Hannun et al., 1985), both of which occur extensively in the plasma membrane (Hannun et al., 1985). In addition, the enzyme shows a marked increase in its phosphorylating activity upon addition of diacylglycerol (DG) to the lipid matrix surrounding the membrane-associated (i.e., translocated) form of the enzyme (Hannun et al., 1985). For maximal activity, however, Ca^{2+} (at a concentration of $\sim 5\ \mu M$) is also required in the microenvironment of the enzyme (Hannun et al., 1985). The phorbol esters and cis-fatty acids also pharmacologically activate PKC (Morimoto et al., 1988). This activation occurs via high-affinity binding near the DG binding site on the enzyme (Huang, 1989).

Neuroanatomical Localization

The high affinity of certain phorbol esters for PKC has also led to mapping studies in which ^{3}H-labeled phorbol-12,13-dibutyrate ([^{3}H]PDBu) has been used as a radioligand (Worley et al., 1986a; Worley et al., 1986b; Huang et al., 1988; el-Fakahany et al., 1988; Onodera et al., 1989; Olds et al., 1989; Olds et al., 1990). The results of these autoradiographic mapping studies have been largely confirmed by less quantitative immunohistochemical studies in which antibodies specific to isozymes of PKC were used. In general, PKC in the mammalian nervous system is highly enriched in the superficial layers of the neocortex, the hippocampus, and the Purkinje cells of the cerebellum (Huang FL et al., 1989; Huang KP et al., 1989b; Roth et al., 1989; Saito et al., 1989; Stichel et al., 1988; Huang FL et al., 1988; Huang KP et al., 1988; Saito et al., 1988). Immunohistochemical studies at the ultrastructural level have shown that the enzyme is associated with the plasma membrane in axons and dendrites and at the somata (Yoshida et al., 1988). Some studies have also shown that at least one isozyme is associated with the Golgi apparatus (Saito et al., 1989).

ROLES OF PKC

Protein Substrates

Several important substrates for PKC have been discovered in vertebrate and invertebrate brain. These include PKC itself, which autophosphorylates on threonine residues (Schwartz et al., 1987), and an 87-kD protein, MARKS, which has been characterized and found to be heavily myristoylated (Ouimet et al., 1990). The 87-kD protein is found throughout the brain, but is enriched in the piriform and entorhinal cortices, the amygdala, intralaminar thalamic nucleii, the hypothalamus, and many aminergic nucleii. Ultrastructural analysis has shown this protein to be localized in axons and small dendrite endings, but not in somata and large dendrites (Ouimet et al., 1990). Recent evidence suggests that the phosphorylation of this protein substrate for PKC may be specifically decreased in Alzheimer's disease (Cole et al., 1988).

Another major substrate for PKC is the B50 protein (also referred to as GAP43, F1), which is specifically phosphorylated by PKC at serine, position 41 (Coggins et al., 1989). This substrate protein occurs in presynaptic nerve endings in the central nervous system (CNS). It is especially enriched in the mammalian hippocampus (Routtenberg, 1986), and its phosphorylation has been shown to parallel long-term-potentiation (LTP) (Lovinger et al., 1986; Akers and Routtenberg, 1987; Lovinger and Routtenberg, 1988). It has also been observed that B50 forms gradients such that its phosphorylation state increases along the cortical visual processing pathways of monkeys (Nelson et al., 1987) and in growth cones of regenerating axons (Van Lookeren et al., 1989).

Finally, a PKC substrate of 20 kD that is particularly intriguing has been described in canine cerebral cortex (Suzuki and Siekevitz, 1989), in the rabbit hippocampus, and in the nudibranch *Hermissenda crassicornis* (Neary et al., 1981; Nelson et al., 1990; Nelson et al., personal communication). In the canine cerebral cortex, this substrate has been shown to be densely localized in postsynaptic-densities fractions. In *Hermissenda,* this substrate (cp20) shows learning-specific increases in its degree of phosphorylation, reduces K^+ ionic current in a manner that mimics Pavlovian conditioning, and manifests a GTPase activity (Nelson et al. 1990). The potential identity between the canine, rabbit, and molluscan substrates still remains to be established.

Cellular Signal Transduction: PKC at the Interface Between the Cell and its Immediate Environment

The active phorbol esters were initially characterized as tumor promoters (Hecker, 1978; Castagna et al., 1982) and, after the initial identification of PKC as the phorbol ester receptor (Ashendel et al., 1983), it became clear that the enzyme itself plays a major role in the cellular transformation that underlies oncogenesis (for a review see Nishizuka et al., 1988). Subsequently it became evident that PKC is also important in cellular growth, secretion, and adhesion (for a review see Nishizuka et al., 1988). These roles reflect the more

general role of PKC in signal transduction. Extrinsic signals, such as phorbol esters or endogenous growth factors, are able to effect a kind of cellular memory (i.e., very long-term changes in the cell) by the PKC activation process. These extrinsic signals are also highly regulated by cross-talk between PKC and other second and tertiary messenger systems within the cell (Nishizuka, 1988).

PKC AND LEARNING

Learning Operationally Defined

Learning can be defined operationally as a relatively permanent change in an animal's behavior as a result of its experience. At its most "atomic" level, learning consists of the formation of associations between stimuli that occur together or at least near one another in time. The associative nature of learning was described initially by Pavlov in 1910 and later by Hebb (Hebb, 1949). Over the years, classical (associative) conditioning has been used by some investigators to assess learning and its correlates in a wide variety of animals ranging from the marine snail to mammals (Olds, 1972; Olds et al., 1972; Olds, 1975; Alkon, 1980; Alkon et al., 1982; Takenda and Alkon, 1982; Crow and Offenbach, 1983; Crow, 1983; Crow, 1985a,b; Farley and Auerbach, 1986; Collin et al., 1988; Matzel et al., 1989).

Injection Studies in Hermissenda crassicornis

The first suggestion that PKC might play an important role in the neuronal modifications that underlie associative learning came from experiments on the marine nudibranch, *Hermissenda crassicornis*. *Hermissenda* can be classically conditioned to associate light with rotation, leading to the development of a new conditioned response (shortening of the animal's foot). The biological substrate for this conditioned response has been demonstrated to reside within the type B photoreceptor, which lies "strategically" at the convergence point of information flow between the visual and vestibular sensory pathways (for a review see Alkon, 1989). Injection of PKC directly into the B photoreceptor, concurrent with the delivery of a Ca^{2+} load (via light flash) mimicked the effects of Pavlovian conditioning on these cells (Alkon et al., 1988). Specifically, the Ca^{2+}-dependent K^+ current showed a profound decrease in conductance, as had been shown previously in animals that had received repeated paired light and rotation stimuli, but not in control animals (Alkon, 1984; Alkon et al., 1985).

The involvement of PKC in *Hermissenda* classical conditioning was recently confirmed autoradiographically in mapping studies. In this case, Pavlovian conditioning produced an increase in [^{3}H]PDBu binding, as assessed by computerized silver grain image analysis of these same B photoreceptors that had previously been shown to undergo biophysical changes with learning (McPhie et al., 1989). This modification in response to conditioning was behaviorally specific, in that animals in the control group did not exhibit it, and was also anatomically specific at the cellular level since only cells previously demonstrated to be crucial to the development of the conditioned response showed the change.

Activity Measurements in Rabbits

In parallel with the results from the invertebrate studies, a steady-state increase in PKC activity was found to be associated with the membrane in the CA1 hippocampal cell field of rabbits that had received three days of Pavlovian conditioning of the nictitating membrane (Bank et al., 1988). This sustained increase lasted as long as 24 h after the last training trial. It has been previously demonstrated that a steady-state decrease in a specific K^+ current (I_{AHP}) in CA1 pyramidal cells occurs in similarly conditioned rabbits but not in control animals (Disterhoft et al., 1986; Loturco et al., 1988; Coulter et al., 1989; Sanchez and Alkon, in preparation) and that PKC-activating phorbol esters can mimic the effect of Pavlovian conditioning on the I_{AHP} (Alkon et al., 1986). These results together suggested an important role for PKC in associative memory storage within the hippocampus. Furthermore, voltage clamp studies have indicated that the I_{AHP} is subserved by a K^+ current (Sanchez and Alkon, in preparation). Thus, the ionic current modified by associative conditioning is similar in *Hermissenda* and the rabbit.

Quantitative Autoradiographic Studies

The advent of computerized image analysis and the development of high-affinity radioligands for PKC (Worley et al., 1986) have made it possible to map the distributional changes in this enzyme after associative conditioning. In our laboratory, we have used [^{3}H] PDBu quantitative autoradiography to study membrane-associated PKC in rabbits that had received 3 days of Pavlovian conditioning trials. Image analysis revealed a dramatic increase in membrane-associated PKC in the CA1 region of the hippocampus of conditioned animals but not control animals (Fig. 1). This change in the distribution of the enzyme within the hippocampus was both long-lasting and dynamic. While the increase was primarily localized in the area of the CA1 pyramidal cell somata 24 h after conditioning, the area of increased binding shifted to the basilar dendrites 72 h after conditioning (Olds et al., 1989).

In additional studies, the same methodology was used to study the initial acquisition of the conditioned

THE NEW BIOLOGIST, Vol. 3, No. 1 (January 1991: 27-35)

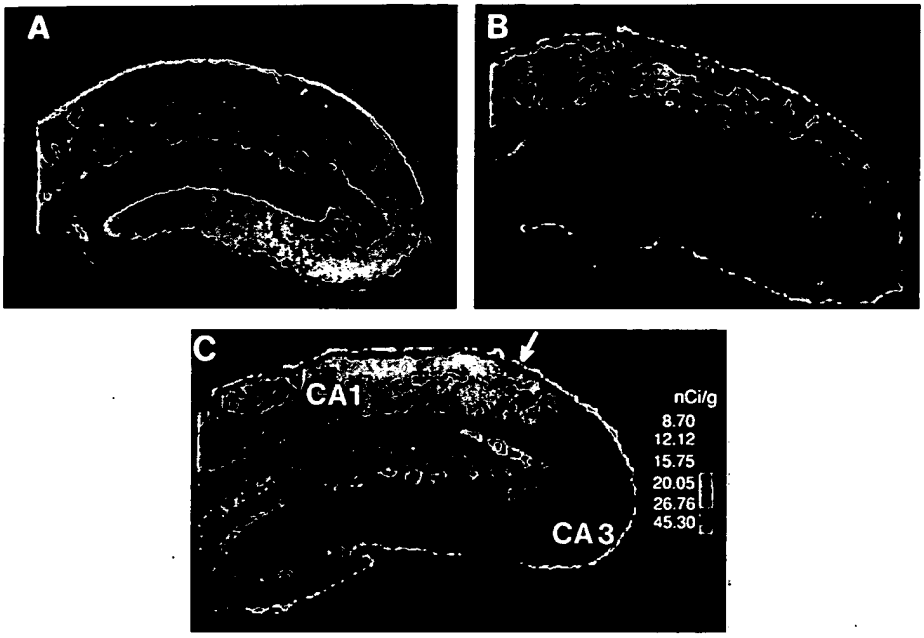

Figure 1. Computer-generated pseudocolor images of PKC distribution in hippocampi from three representative rabbits.

The rabbits were treated as follows: (A) classically conditioned (90 trials paired tone and periorbital electric shock) for 3 days and then allowed a 24 h retention period; (B) pseudoconditioned in such a way that the same number of stimuli were delivered but in an explicitly unpaired manner; or (C) kept in their home cages for 4 days (control group). In all sections, the region of interest (indicated by the arrow) was determined in a manner such that the experimenter did not know which experimental group the data were from. Classically conditioned animals showed a 49% and 43% increase in PKC within the CA1 cell field, as assessed by [^{3}H]PDBu binding, over the pseudoconditioned and control groups, respectively ($F = 8.716$, $p < 0.01$, one way analysis of variance, $n = 5$ in each group). The color bar provides quantitative calibration for all three images. Adapted from Olds et al. (1990), Science 245:866-869.

response in rabbits. In contrast to the CA1-specific increase in membrane-associated PKC seen 24 h and 72 h after three days of classical conditioning, rabbits studied after 80 conditioning trials showed an increase in PKC membrane association specific to the stratum oriens of CA3 but not CA1 (Scharenberg et al., 1990).

A hippocampal-specific change in PKC membrane association was also seen in rats that had received discrimination training in a water maze procedure, but not in control animals (Fig. 2) (Olds et al., 1990). In this case, the binding of [^{3}H]PDBu was significantly reduced in the CA3 region of animals from the discrimination groups (SD and CD) compared to that observed in the CA3 region of the rats in the NC and SNS control groups. Thus, both spatial and cued discrimination procedures produced a significant decrease in the binding of PKC in the CA3 cell field. Both tasks required an intact hippocampus. What was extraordinary about this finding in relation to the previous result in rabbit hippocampus was the involvement of hippocampal PKC in a different species that was performing a completely different sort of task. This suggests that the enzyme's role in memory storage is not simply an artifact of the learning task but reflects a more generalized involvement of PKC in a biological mechanism. This finding has recently been supported by work with inbred mouse mutants (Wehner et al., 1990a) in which mice with superior spatial-learning ability were shown to have significant changes in hippocampal PKC activity.

PKC'S ROLE IN OTHER MODEL SYSTEMS

Dietary Activation of PKC and Learning

Recently, Routtenberg and co-workers have reported a significant improvement in the maze learning performance of rats fed cis-fatty acids. This improvement resulted in an increase in the PKC-dependent phosphorylation of B50 in hippocampus (Wong et al., 1989).

PKC and Alzheimer's Disease

Alzheimer's disease (AD) is characterized by a decline in cognitive functions (especially memory) in combination with neuronal cell death in the basal forebrain, neocortex, and hippocampus over a relatively long period of time (Katzman, 1986). Recent analysis of human tissue obtained post mortem from AD patients and age-matched controls has revealed a striking decrease in the levels of PKC found in these

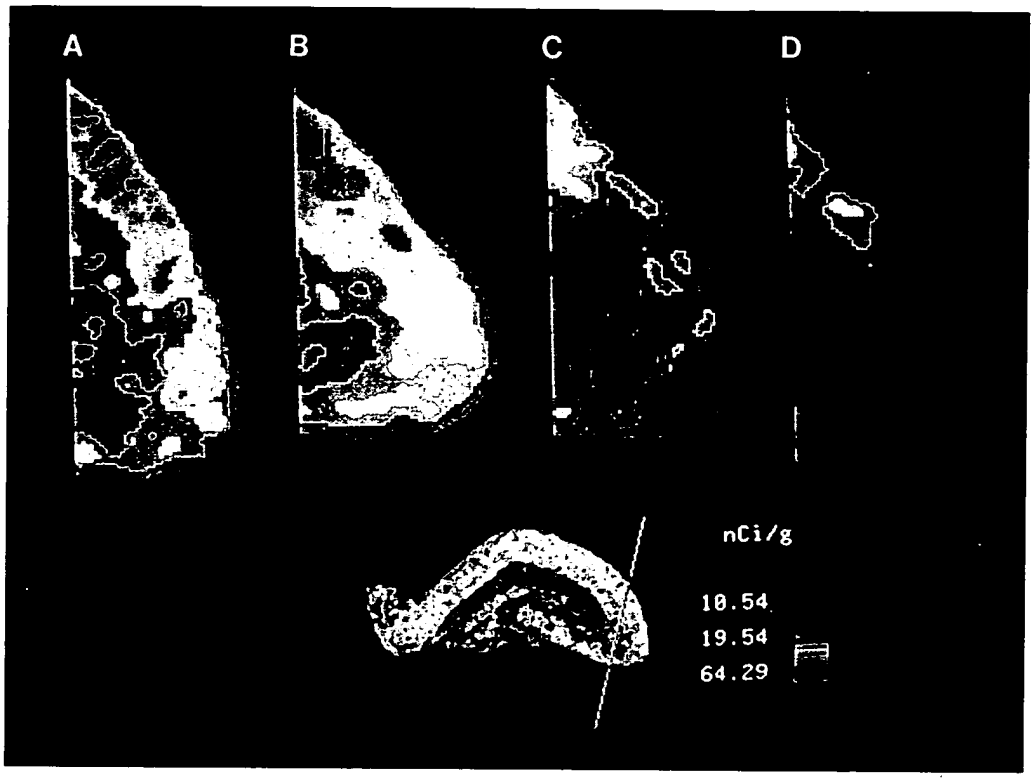

Figure 2. Computer-generated pseudocolor images of membrane-associated PKC distribution in representative CA3 regions from the dorsal hippocampi.

Four groups of rats were trained in a water maze discrimination procedure. They are represented as: (A) control (c); (B) swim no stimuli (SNS); (C) spatial discrimination (SD); and (D) cue discrimination (CD). (A) Control group. Each rat was handled several times on each of three consecutive days and then remained in its cage for the duration of the experiment. Baseline levels of [^{3}H]PDBu binding to PKC were observed. (B) SNS group. These rats had the same motor experience as rats in the two discrimination groups but did not receive discrimination training in that the platforms, platform stimuli, and tank stimuli were removed from the tank. Each SNS rat was randomly yoked to a rat in the spatial discrimination (SD, n = 4) or cue discrimination (CD, n = 4) condition. The swim time for each trial was determined by the response time of the rat in the SD or CD condition to which the SNS rat was yoked. Thus, the swim time for each SNS rat was gradually reduced across trials to that of a rat in the appropriate (SD or CD) group. In each trial, the rat, when released from the appropriate start location, was allowed to swim in the tank. After the predesignated amount of time, the rat was removed from the tank. Each rat was given 9 blocks of 12 trials for a total of 108 trials with an intertrial interval of 8 min. (C) SD group. These rats received training in cognitive mapping. One topographic relationship (TR) among the tank stimuli, which remained constant, was chosen randomly and used for all SD rats throughout the test. The platform stimulus (white or striped) on the stable platform (SP) varied from trial to trial. Thus, only the tank stimuli were relevant for the SD rats identifying the stable platform; the platform stimuli were irrelevant. The tank stimuli were arranged on the tank in one of six possible rotations, and the grid of possible platform locations was rotated to maintain a constant TR between the SP and the tank stimuli. The six rotations were randomized between trials; however, the same rotation was never used on consecutive trials. The other platform stimulus was placed in one of the 11 remaining positions in the tank. (D) CD group. The rats in this group learned a cue discrimination. The TR among the tank stimuli and between the tank stimuli and the SP was varied from trial to trial. The platform stimulus on the SP was constant. Thus, only the platform stimuli were relevant for the CD rats identifying the SP; the tank stimuli were irrelevant. Five specified TRs among the tank stimuli were counterbalanced so that each TR appeared once in every block of five trials. For each trial, the tank stimuli were arranged according to the specified TR, independent of the location of the SP. The white platform stimulus was attached to the top of the SP, which was placed in one of the 12 locations, and the striped platform stimulus was placed in one of the 11 remaining positions. The region of interest of each digitized autoradiographic brain section is represented in the inset. Each dorsal hippocampus was individually magnified by a factor of two and subjected to a low frequency 3 × 3 convolution matrix filter operation to reduce high frequency autoradiographic artifact prior to sampling. Values for each CA3 region of interest were averaged for all sections from an animal and these average values were then compared between the groups using one way analyses of variance performed with the Systat statistical analysis system (Systat Inc., La Jolla, CA). The color bar in the lower right provides quantitative calibration for all four images. Reprinted from Olds et al. (1990), courtesy of the Journal of Neuroscience.

tissues, as measured by radioactive phorbol ester binding (Cole et al., 1988) as well as by quantitative immunohistochemistry (Masliah et al., 1990). This decrease does not simply reflect neuronal cell death, since fibroblasts derived from AD patients also have significantly reduced PKC compared to those from control patients (Huynh et al., 1989). What is intriguing about the most recent clinical results is that the

THE NEW BIOLOGIST, Vol. 3, No. 1 (January 1991: 27-35)

decrease seems to be specific to the βII isozyme of PKC in tissue from AD human hippocampus and cortex when compared to controls (Masliah et al., 1990). Thus, the amount of a specific isozyme of PKC has been demonstrated to be significantly decreased in tissues from patients whose disease involves not only memory, but also a specific neuropathology in the hippocampus.

Long-Term Potentiation

In addition to its potential role in associative learning, PKC has also been shown to be involved in a laboratory model of synaptic plasticity known as long-term potentiation (LTP). LTP refers to the long-term enhancement of the excitatory post-synaptic potential produced by rapid tetanic stimulation of presynaptic axons both within the hippocampus and, as recently shown, in other neuroanatomic structures (Bliss and Lomo, 1973; Levy and Steward, 1983). Routtenberg and his colleagues observed increases in the phosphorylation of the PKC substrate protein B50 that parallel the development of LTP (Routtenberg 1986; Lovinger et al., 1988; Linden et al., 1989). Furthermore, application of phorbol esters to hippocampal slices prolonged the maintenance of LTP (Routtenberg et al., 1986; Malenka et al., 1986; Malenka et al., 1987). It was even suggested that the effects of phorbol esters mimicked entirely the synaptic enhancement of LTP (Malenka et al., 1986) since, in initial studies, the application of phorbol ester to hippocampal slices resulted in a saturation of synaptic enhancement such that further tetanic stimulation would elicit no further LTP.

Supporting the theory that PKC is involved at least in the maintenance of LTP, it was shown that pharmacological blockers of PKC such as H7, polymyxin B, sphingosine, and melitin inhibit the maintenance of LTP after its PKC-independent initiation (Malenka et al., 1986; Lovinger et al., 1988). However, all of these inhibitors may have nonspecific effects on other kinases, as well as on PKC.

PKC as a Multifunctional Integrator of Cotemporal Synaptic Input

The above discussion inevitably leads to the question of the role of PKC in the chain of molecular events in neural elements that result in memory storage. We have proposed a multifunctional model that is described below (Alkon 1989; Olds et al., 1989).

Stage I: Dendritic activation of PKC. In the first stage of the proposed model for memory storage, signals from the immediate environment of the animals are filtered for salience on a subconscious level. This filtering process takes into account physiological variables such as the current drive state of the animal (e.g., is it sated?). Thus, a tone is perceived to be important, or salient, to a rabbit only if it occurs within the appropriate environmental or behavioral milieu. The neurobiological substrate for such filtering of stimuli might involve either the cholinergic or noradrenergic systems of the mammalian nervous system, since both of them project widely not only onto the cells of the hippocampus, but also onto the entire neocortex (Lehman et al., 1984). Furthermore, both the cholinergic and noradrenergic systems can affect PKC (Hashimoto et al., 1988; Agopyan et al., 1989). Signals that pass through this filtering process eventually converge on the dendritic branches of certain neurons that, for heuristic purposes, we call neural storage elements (NSE's). Signals that occur together, or nearly together, in time activate multiple excitatory amino acid receptors, including quisqualate and *N*-methyl-D-aspartate (NMDA), which together (via phospholipase C and A and also via the NMDA channel) provide the necessary activational cofactors for PKC. Hypothetically, this activated, or membrane-associated, form of PKC becomes localized in the area (or patch) of the NSE membrane that received the two signal inputs (Fig. 3, stage I) and no longer depends on other cofactors for further activation (Nelsestuen and Bazzi, 1989). In other words, once PKC has become associated with the membrane (because of complexing with its cofactors) it is constitutively activated. This initial stage I activation corresponds to the acquisition data described by Scharenberg et al. (1990) in CA3 stratum oriens. Other synaptic input onto the NSE (not carrying information about salient sensory stimuli) may play a critical role in gating this PKC activation.

The association and localization of PKC with the NSE described above leads to PKC-mediated phosphorylation of Ca^{2+}-dependent K^+ channels, either directly or possibly via phosphorylation of intermediate proteins such as the recently discovered cp20 (Nelson et al., 1990). The phosphorylation of these K^+ channels might then cause their inactivation. This stage renders the patch of NSE more electrically responsive to both of the initial input signals (either together or alone) and, in effect, indexes an association between them. Thus, after stimulus pairing in stage I and in response to both of the original synaptic inputs, the patch of NSE generates a post-synaptic potential that is significantly greater than the synaptic potential generated by either input alone before conditioning. Since the NSE has been potentiated, one of the two stimuli will now substitute for the previous effect of both stimuli at the same time.

Stage II: PKC activation of retro-messages. In stage II, consolidation (Fig. 3, stage II), the well-described process of PKC downregulation (Nishizuka, 1988), begins to occur in the patch of activated NSE. This may be due to proteolytic degradation of the activated PKC and to the dephosphorylation of either the K^+ channels

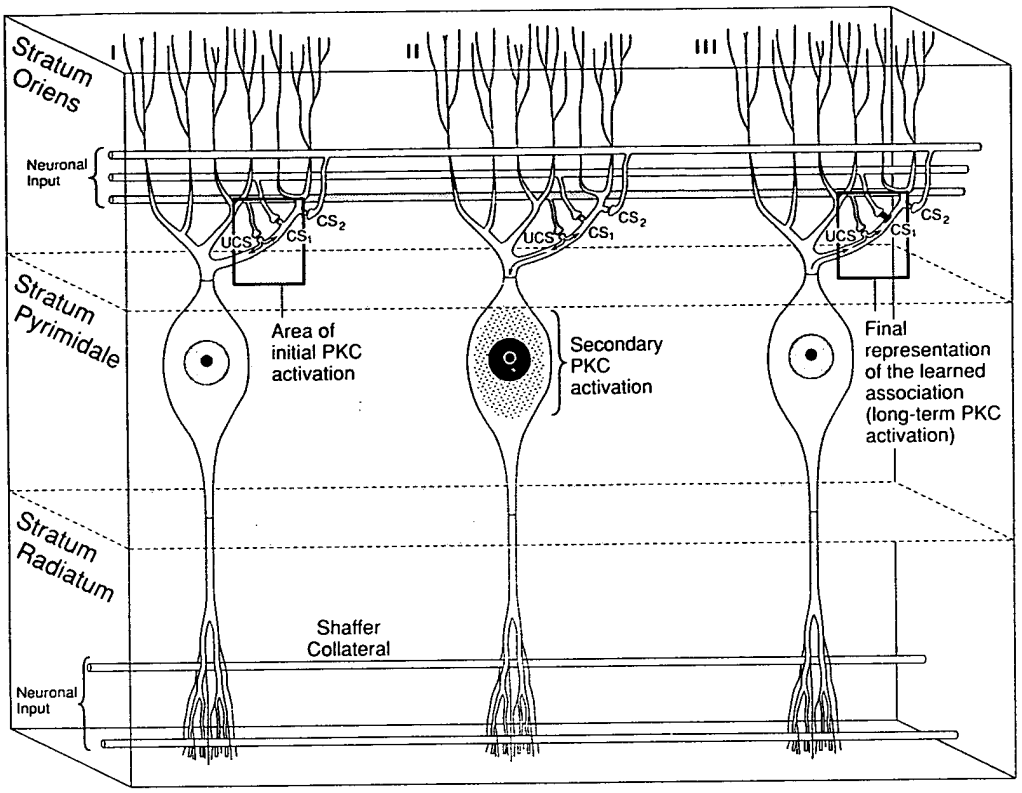

Figure 3. Schematic depiction of pyramidal cells in the CA1 region of the hippocampus illustrating a sequential activation of PKC within different intracellular spatial domains.

(Stage I) Initial cotemporal input of both a conditioned stimulus (CS_1) and unconditioned stimulus (UCS) results in local activation of PKC at the postsynaptic neural storage element (NSE; this is represented by stippling). **(Stage II)** After the initial stimulation of PKC at the NSE, a sequence of cellular events causes alterations in cellular transport mechanisms and, in addition, results in an apparent increase in membrane-associated PKC associated with cell body organelles. **(Stage III)** In the last stage of "consolidation," specifically targeted, newly synthesized proteins (possibly including PKC) make their way back to the dendritic region of the initially activated NSE, resulting in a long-term change in the biophysical characteristics of the NSE in response to new inputs via the CS_1 input pathway. CS_2 represents input from a temporally unpaired stimulus.

or the intermediate target proteins by phosphatases. However, the same PKC downregulation process that results essentially in the homeostatic reversal of stage I, causes an alteration in neuronal transport mechanisms. This alteration in neuronal transport mechanisms within the cell somata may be caused by either a molecular or an electrical retro-message such that specific molecules (possibly including new PKC molecules and/or cp20) are targeted and then subsequently transported to the NSE (Fig. 3, stage III). This stage of memory storage corresponds to the 72-hour data on hippocampal PKC distribution in classically conditioned rabbits, where [^{3}H]PDBu binding shifted from the area of the stratum pyrimidale to the area of the stratum oriens (Olds et al., 1990). This stage is also supported by recent findings in our laboratory that injection of cp20 into invertebrate neurons profoundly affects axonal transport (Alkon et al., in preparation) and by immunohistochemical studies showing the β PKC isozyme to be associated with the Golgi apparatus (Saito et al., 1989). This de novo insertion of new proteins into the original NSE renders the original biophysical change that occurred in stage I more permanent.

Stage III: Genomic alterations. In the final stage of memory storage (not illustrated schematically in Fig. 3), the activation of certain oncogenes may occur leading to the transcriptional upregulation of specific genes. In support of this hypothesis, PKC activation has been shown to activate the binding of Fos/Jun to the AP1 promoter site of some genes (Auwerx et al., 1990). The protein produced by such genomic regulation could be transported in a manner similar to the transport of proteins in stage II to the NSE, thus resulting in much longer-term changes.

CONCLUDING REMARKS

We have reviewed some of the evidence for the involvement of PKC in associative learning. This evidence comes from studies on animals that are widely

separated in phylogeny but that share the characteristic of being able to show robust associative learning. The changes in PKC distribution that seem to parallel associative learning occur in nerve cells that have been independently demonstrated to be at critical convergence points in the association process (i.e., at the juncture of the conditioned stimulus and unconditioned stimulus pathways in the parlance of Pavlovian conditioning). A variety of methodologies have been used to track the activity and distribution of PKC within these nerve cells, and taken together the data seem to suggest that PKC has a major role in the memory storage process.

REFERENCES

Agopyan N, Krnjevic C, Leblond J (1989): Mediation of acetylcholine's excitatory actions in central neurons. Experientia Suppl 57:77-87

Alkon DL (1980): Membrane depolarization accumulates during acquisition of an associative behavioral change. Science 210:1375-1376

Alkon DL (1984): Changes of membrane currents during learning. J Exp Biol 112:95-112

Alkon DL (1989): Memory storage and neural systems. Scientific American, July: 42-50

Alkon DL, Kubota M, Neary JT, Naito S, Coulter D, Rasmussen H (1986): C-kinase activation prolongs Ca^{2+}-dependent inactivation of K^+ currents [published erratum appears in Biochem Biophys Res Commun 1986 Oct 30;140(2):774]. Biochem Biophys Res Commun 134:1245-1253

Alkon DL, Lederhendler I, Shoukimas JJ (1982): Primary changes of membrane currents during retention of associative learning. Science 215:693-695

Alkon DL, Naito S, Kubota M (1988): Regulation of Hermissenda K^+ channels by cytoplasmic and membrane-associated C-kinase. J Neurochem 51:903-917

Alkon DL, Rasmussen H (1988): A spatial-temporal model of cell activation. Science 239:998-1005

Alkon DL, Sakakibara M, Forman R, Harrigan J, Lederhendler I, Farley J (1985): Reduction of two voltage-dependent K^+ currents mediates retention of a learned association. Behav Neural Biol 44:278-300

Auwerx J, Staels B, Sassone-Corsi P (1990): Coupled and uncoupled induction of fos and jun transcription by different second messengers in cells of hematopoietic origin. Nucleic Acids Res 18:221-228

Bank B, DeWeer A, Kuzirian AM, Rasmussen H, Alkon DL (1988): Classical conditioning induces long-term translocation of protein kinase C in rabbit hippocampal CA1 cells [published erratum appears in Proc Natl Acad Sci USA 1988 85:5344]. Proc Natl Acad Sci USA 85:1988-1992

Brumfeld V, Lester DS (1990): Protein kinase C penetration into lipid bilayers. Achives Biochem Biophys 277:318-323

Coggins PJ, Zwiers H (1989): Evidence for a single protein kinase C-mediated phosphorylation site in rat brain protein B-50. J Neurochem 53:1895-1901

Cole G, Dobkins KR, Hansen LA, Terry RD, Saitoh T (1988): Decreased levels of protein kinase C in Alzheimer brain. Brain Res 452:165-170

Collin C, Ikeno H, Harrigan JF, Lederhendler I, Alkon DL (1988): Sequential modification of membrane currents with classical conditioning. Biophys J 54:955-960

Coulter DA, Lo Turco JJ, Kubota M, Disterhoft JF, Moore JW, Alkon DL (1989): Classical conditioning reduces amplitude and duration of calcium-dependent afterhyperpolarization in rabbit hippocampal pyramidal cells. J Neurophysiol 61:971-981

Crow T (1983): Conditioned modification of locomotion in Hermissenda crassicornis: analysis of time-dependent associative and nonassociative components. J Neurosci 3:2621-2628

Crow T (1985a): Conditioned modification of phototactic behavior in Hermissenda I. Analysis of light intensity. J Neurosci 5:209-214

Crow T (1985b): Conditioned modification of phototactic behavior in Hermissenda II. Differential adaptation of B-photoreceptors. J Neurosci 5:215-223

Crow T, Offenbach N (1983): Modification of the initiation of locomotion in Hermissenda: behavioral analysis. Brain Res 271:301-310

Disterhoft JF, Coulter DA, Alkon DL (1986): Conditioning-specific membrane changes of rabbit hippocampal neurons measured in vitro. Proc Natl Acad Sci USA 83:2733-2737

el-Fakahany EE, Alger BE, Lai WS, Pitler TA, Worley PF, Baraban JM (1988): Neuronal muscarinic responses: role of protein kinase C. FASEB J 2:2575-2583

Farley J, Auerbach S (1986): Protein Kinase C activation induces conductance changes in Hermissenda photoreceptors like those seen in associative learning. Nature 319:220-223

Hannun YA, Loomis CR, Bell RM (1985): Activation of protein kinase C by Triton X-100 mixed micelles containing diacylglycerol and phosphatidylserine. J Biol Chem 260:10039-10043

Hashimoto S, Suntoh H, Taniyama K, Tanaka C (1988): Role of protein kinase C in the vesicular release of acetylcholine and norepinephrine from enteric neurons of the guinea pig small intestine. Jpn J Pharmacol 48:377-385

Hecker E (1978): A Comprehensive Survey, in Slaga TJ, Sivak A, Boutwell RK (eds) Carcinogenisis, New York, Raven Press, Vol. 2, pp 11-48

Hidaka H, Tanaka T, Onoda K, Hagiwara M, Watanabe M, Ohta H, Ito Y, Tsurudome M, Yoshida T (1988): Cell type-specific expression of protein kinase C isozymes in the rabbit cerebellum. J Biol Chem 263:4523-4526

Huang FL, Yoshida Y, Nakabayashi H, Friedman DP, Ungerleider LG, Young WS III, Huang KP (1989): Type I protein kinase C isozyme in the visual-information-processing pathway of monkey brain. J Cell Biochem 39:401-410

Huang FL, Yoshida Y, Nakabayashi H, Huang KP (1987): Differential distribution of protein kinase C isozymes in the various regions of brain. J Biol Chem 262:15714-15720

Huang FL, Yoshida Y, Nakabayashi H, Young WS III, Huang KP (1988): Immunocytochemical localization of protein kinase C isozymes in rat brain. J Neurosci 8:4734-4744

Huang KP (1989): The mechanism of protein kinase C activation. Trends Neurosci 12:425-432

Huang KP, Huang FL, Nakabayashi H, Yoshida Y (1988): Biochemical characterization of rat brain protein kinase C isozymes. J Biol Chem 263:14839-14845

Huang KP, Huang FL, Nakabayashi H, Yoshida Y (1989): Expression and function of protein kinase C isozymes. Acta Endocrinol (Copenh) 121:307-316

Huynh TV, Cole G, Katzman R, Huang K-P, Saitoh T (1989): Reduced PK-C immunoreactivity and altered protein phosphorylation in Alzheimer's disease fibroblasts. Arch Neurol 43:1195-1199

Katzman R (1986): Alzheimer's disease. N Engl J Med 314:964-973

Lehman J, Struble R, Antuons P, Coyle J, Cork L, Price D (1984): Regional heterogeneity of choline acetyltransferase activity in the primate neocortex. Brain Res 322:361-364

Lester DS, Orr N, Brumfeld V (1990): Structural distinction between soluble and particulate protein kinase C species. J Protein Chem 9:209-220

Linden DJ, Routtenberg A (1989): The role of protein kinase C in long-term potentiation: a testable model. Brain Res Rev 14:279-296

LoTurco JJ, Coulter DA, Alkon DL (1988): Enhancement of synaptic potentials in rabbit CA1 pyramidal neurons following classical conditioning. Proc Natl Acad Sci USA 85:1672-1676

Lovinger DM, Routtenberg A (1988): Synapse-specific protein kinase C activation enhances maintenance of long-term potentiation in rat hippocampus. J Physiol (Lond) 400:321-333

Makowske M, Ballester R, Cayre Y, Rosen OM (1988): Immunochemical evidence that three protein kinase C isozymes increase in abundance during HL-60 differentiation induced by dimethyl sulfoxide and retinoic acid. J Biol Chem 263:3402-3410

Malenka RC, Ayoub GS, Nicoll RA (1987): Phorbol esters enhance transmitter release in rat hippocampal slices. Brain Res 403:198-203

Malenka RC, Madison DV, Andrade R, Nicoll RA (1986): Phorbol esters mimic some cholinergic actions in hippocampal pyramidal neurons. J Neurosci 6:475-480

Masliah E, Cole G, Shimohama S, Hansen L, DeTeresa R, Terry RD, Saitoh T (1990): Differential involvement of protein kinase C isozymes in Alzheimer's Disease. J Neurosci 10:2113-2124

Matzel LD, Schreurs B, Lederhendler I, Alkon DL (1990): Acquisition of conditioned associations in Hermissenda: Additive effects of contiguity and the forward interstimulus interval. Behavioral Neuroscience 104:597-606

McPhie DL, Olds JL, Sanchez-Andres JV, Alkon DL (1989): Isolated pharmacological activation of three neurotransmitter systems produces differential translocation of PKC in the rabbit hippocampus. Society for Neuroscience Meeting, 15:505-505. (Abstract)

Morimoto YM, Nobori K, Edashige K, Yamamoto M, Kobayashi S, Utsumi K (1988): Activation of protein kinase C by fatty acids and its dependency on Ca^{2+} and phospholipid. Cell Struct Funct 13:45-49

Nakabayashi H, Huang KP (1988): Monoclonal antibodies against type II rat brain protein kinase C. J Biol Chem 263:298-304

Neary JT, Crow T, Alkon DL (1981): Change in a specific phosphoprotein band following associative learning in Hermissenda. Nature 293:658-660

Nelsestuen GL, Bazzi MD (1989): In vitro properties of protein kinase C suggest an accumulating and long-term regulation, in Cell Activation and Signal Initiation: Receptor and Phospholipase Control of Inositol Phosphate, PAF and Eicosanoid Production, New York, Alan R. Liss, pp 253-266

Nelson RB, Friedman DP, O'Neill JB, Mishkin M, Routtenberg A (1987): Gradients of protein kinase C substrate phosphorylation in primate visual system peak in visual memory storage areas. Brain Res 416:387-392

Nelson TJ, Collin C, Alkon DL (1990): Isolation of a G Protein That is Modified by Learning and Reduces Potassium Currents in Hermissenda. Science 247:1479-1483

Nishizuka Y (1988a): The molecular heterogeneity of protein kinase C and its implications for cellular regulation. Nature 334:661-665

Olds J (1972): Learning and the hippocampus. Rev Can Biol 31:Suppl:215-Suppl:238

Olds J (1975): Unit recordings during Pavlovian conditioning. UCLA Forum Med Sci:343-371

Olds J, Disterhoft JF, Segal M, Kornblith CL, Hirsh R (1972): Learning centers of rat brain mapped by measuring latencies of conditioned unit responses. J Neurophysiol 35:202-219

Olds JL, Anderson ML, McPhie DL, Staten LD, Alkon DL (1989): Imaging of memory-specific changes in the distribution of protein kinase C in the hippocampus. Science 245:866-869

Olds JL, Golski S, McPhie DL, Olton D, Mishkin M, Alkon DL (1990): Discrimination learning alters the distribution of protein kinase C in the hippocampus of rats. J Neurosci 10:3707-3713

Onodera H, Araki T, Kogure K (1989): Protein kinase C activity in the rat hippocampus after forebrain ischemia: autoradiographic analysis by [3H]phorbol 12,13-dibutyrate. Brain Res 481:1-7

Ouimet CC, Wang JK, Walaas SI, Albert KA, Greengard P (1990): Localization of the MARCKS (87 kDa) protein, a major specific substrate for protein kinase C, in rat brain. J Neurosci 10:1683-1698

Pavlov IP (1910): Conditioned Reflexes (G.V. Anrep, translator and ed.) Oxford University Press, London.

Roth BL, Iadarola MJ, Mehegan JP, Jacobowitz DM (1989a): Immunohistochemical distribution of beta-protein kinase C in rat hippocampus determined with an antibody against a synthetic peptide sequence. Brain Res Bull 22:893-897

Roth BL, Mehegan JP, Jacobowitz DM, Robey F, Iadarola MJ (1989b): Rat brain protein kinase C: purification, antibody production, and quantification in discrete regions of hippocampus. J Neurochem 52:215-221

Routtenberg A (1986): Synaptic Plasticity and protein kinase C, in Gispen WH and Routtenberg A (eds) Progress in Brain Research, New York, Elsevier Science Publishers BV, pp 211-234

Saito N, Kikkawa U, Nishizuka Y, Tanaka C (1988): Distribution of protein kinase C-like immunoreactive neurons in rat brain. J Neurosci 8:369-382

Saito N, Kose A, Ito A, Hosoda K, Mori H, Hirata M, Ogita K, Kikkawa U, Ono Y, Igarashi K, Nishizuka Y, Tanaka C (1989): Immunocytochemical localization of beta-II subspecies of protein kinase C in rat brain. Proc Natl Acad Sci USA 86:3409-3413

Scharenberg AM, Olds JL, Schreurs BG, Craig AM, Alkon DL: PKC redistribution within CA3 stratum oriens occuring during acquisition of NM conditioning in the rabbit. Proc Natl Acad Sci USA (in press)

Schwartz JH. Greenberg SM (1987): Molecular mechanisms for memory: second-messenger induced modifications of protein kinases in nerve cells. Annu Rev Neurosci 10:459-476

Stichel CC, Singer W (1988): Localization of isoenzymes II/III of protein kinase C in the rat visual cortex (area 17), hippocampus and dentate gyrus. Exp Brain Res 72:443-449

Stopfer M, Carew TJ (1988): Development of sensitization in the escape locomotion system in Aplysia. J Neurosci 8:223-230

Suzuki T, Siekevitz P (1989): Properties of a protein kinase C activity in synaptic plasma membrane and postsynaptic density fractions isolated from canine cerebral cortex. J Neurochem 53:1751-1762

Takeda T, Alkon DL (1982): Correlated receptor and motorneuron changes during retention of associative learning of Hermissenda crassicornis. Comp Biochem Physiol [A] 73:151-157

Van Lookeren Campagne M, Oestreicher AB, Van Bergen En Henegowen PMP, Gispen WH (1989): Ultrastructural immunocytochemical localization of B-50/GAP43, a protein kinase C substrate, in isolated presynaptic nerve terminals and neuronal growth cones. J Neurocytol 18:479-489

Wehner JM. Sleight S, Upchurch M (1990): Hippocampal protein kinase C activity is reduced in poor spatial learners. Brain Res 523:181-187

Wong KL, Murakami K, Routtenberg A (1989): Dietary cis-fatty acids that increase protein F1 phosphorylation enhance spatial memory. Brain Res 505:302-305

Woodgett JR. Hunter T (1987): Isolation and characterization of two distinct forms of protein kinase C. J Biol Chem 262:4836-4843

Worley PF. Baraban JM, De Souza EB, Snyder SH (1986a): Mapping second messenger systems in the brain: differential localizations of adenylate cyclase and protein kinase C. Proc Natl Acad Sci USA 83:4053-4057

Worley PF. Baraban JM. Snyder SH (1986b): Heterogeneous localization of protein kinase C in rat brain: autoradiographic analysis of phorbol ester receptor binding. J Neurosci 6:199-207

Yasutomi N (1988): The molecular heterogeneity of protein kinase C and its implications for cellular regulation. Nature 334:661-665

Yoshida Y. Huang FL, Nakabayashi H, Huang KP (1988): Tissue distribution and developmental expression of protein kinase C isozymes. J Biol Chem 263:9868-9873